APPENDIX

Lyophilization

Introduction and Basic Principles

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mysticism has sprung up around the technique .... Such mysticism stems not only from the deceptive complexity of this technology but also from a lack of understanding of the principles that serve as the foundation for the science of lyophilization.

DEFINITION OF LYOPHILIZATION

The coining of the term *lyophilization* is generally attributed to Rey [8] because of the porous nature of the dried product and its "lyophil" characteristic to rapidly reabsorb the solvent and restore the substance to its original state. Although Rey equated the term *lyophilization* with freeze-drying, the latter term has become more common because it is applicable to both aqueous and nonaqueous systems. It is interesting that lyophilization processes are often conducted in freeze-drying equipment, although the descriptive term *lyophilizer* is becoming more prevalent. In this book, the process will be defined as lyophilization, and the equipment will still be referred to as a freeze-dryer.

Although the steps in the lyophilization process outlined by Rey [8] have been generally accepted, only recently has a definition been given to the term *lyophilization* [9]. In its simplest form, *lyophilization* is defined as a stabilizing process in which the substance is first frozen and then the quantity of the solvent is reduced first by sublimation (*primary drying*) and then by desorption (*secondary drying*) to values that will no longer support biological growth or chemical reactions. Although this definition will be further refined later and the various processes described in greater detail, the key term in the above definition is that lyophilization is a *stabilizing* process.

A *stabilizing process* is one in which the natural kinetic clock of a substance has been greatly altered. Consider a vaccine that will retain 90% of its effectiveness for up to 2 days when stored at 4°C. If by reducing the moisture in the vaccine to levels where the kinetic clock is slowed down to where 1 sec is extended to 1 hr, then the vaccine will have a stability of nearly 20 years rather than just 2 days.

GENERAL DESCRIPTION OF THE PROCESS

The following is a general overview of the lyophilization process and freeze-drying equipment. The intent of this section is merely to familiarize the reader with the basic steps in the process and provide a general description of the equipment. This overview will also provide the reader with an understanding of my selection of topics and the order in which they will be presented in the remaining chapters.

Formulation

A *formulation* is defined as any system containing a solvent that, upon its removal, will enhance the stability of the substance. While the substance to be stabilized can include floral and food products, the majority of lyophilized formulations will consist of biological, biotechnology, diagnostic (in vivo and in vitro), pharmaceutical, and veterinary products. These latter formulations will generally consist of an active constituent and possibly other constituents that are added for stabilizing the

formulation in its liquid state or for therapeutic reasons. In general, a glass container is filled with a specified quantity of the formulation and a special closure designed for lyophilization, as shown in Figure 1.1a, is placed into position.

Freezing

The principal function of the freezing process is to separate the solvent from the solutes. For an aqueous system, the water will form ice crystals, and solutes will be confined to the interstitial region between the ice crystals. The temperature necessary to achieve complete freezing of the formulation will be dependent on the nature of the solvent and other constituents that comprise the formulation. Freezing may be performed in an external freezing unit or on the shelves of the freeze-dryer. An example of the frozen ice–product matrix is given in Figure 1.1b. The freezing

Figure 1.1. Lyophilization in Glass Containers

Fig. 1.1a

Fig. 1.1b

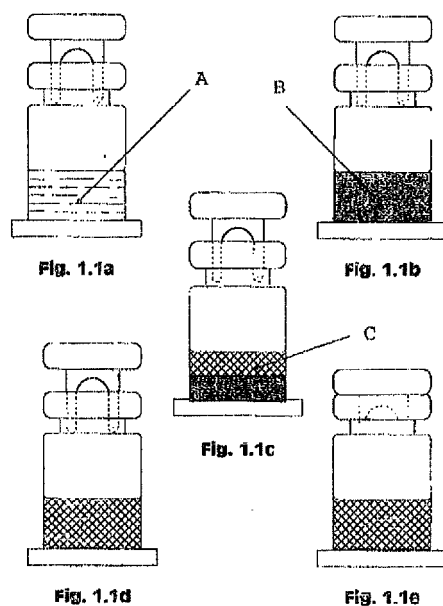Fig. 1.1c

Fig. 1.1d

Fig. 1.1e

Figure 1.1a shows a fill volume of a liquid formulation, denoted by region defined by "A," in a glass container with a lyophilization closure positioned for the drying process; in Figure 1.1b, the frozen ice–product matrix of the formulation is signified by the region "B"; Figure 1.1c illustrates the primary drying process, and the interstitial cake portion is denoted by region "C"; the completion of secondary drying is shown by Figure 1.1d, and the final product with the closure in its stoppered position is illustrated by Figure 1.1e.

shelf. All of the shelves are of a hollow construction that permits the serpentine flow of heat-transfer fluid. The heat-transfer fluid can be chilled to freeze the product or heated to provide the necessary energy for the primary and secondary drying processes. A hydraulic system (G) can move the shelves vertically to provide the necessary force to stopper the closures prior to opening the chamber door (B).

The drying chamber also contains a pressure gauge (H) and is equipped with an insulation covering (I) over the entire chamber surface to prevent heat transfer to the shelves (D) and the trays (F) during the drying process.

The Condenser Chamber

The main function of the condenser chamber is to house the condenser surfaces for the removal of water vapor from the gases that pass from the drying chamber. For the condenser plates to be effective, their operating temperatures must be a minimum of 20°C lower than the product temperature during the primary drying process. Unlike the shelves of the dryer which are chilled by a heat-transfer fluid, the condenser surfaces are generally refrigerated by direct expansion of a refrigerant.

Figure 1.2 illustrates an external condenser system in which the condenser surfaces (J) are housed in a separate insulated vacuum chamber (K). In some dryers, the condenser surfaces are housed in the drying chamber and are referred to as internal condensers.

The Vacuum Pumping System

The vacuum pumping system, in conjunction with the condenser system, provides the necessary pressures for conducting the primary and secondary drying processes. Typical mechanical vacuum pumps used in freeze-dryers are oil lubricated; however, oil-free mechanical pumping systems are available. The vacuum pump (L) shown in Figure 1.2 compresses the noncondensable gases that pass through the condenser chamber (K) and discharges these gases directly into the atmosphere.

PROPERTIES OF LYOPHILIZED MATERIALS

The following is a general description of the key properties of a lyophilized formulation. A more detailed discussion of these properties is given in Chapter 10. This section is designed merely to familiarize the reader with results of the lyophilization of a formulation.

Stability

The principal purpose for conducting a lyophilization process is to enhance the stability of a formulation (i.e., slow down the kinetic clock for the degradation or loss in potency of the active constituent). The dried formulation is considered stable as long as its activity or its potency remains within a given range of values. For example, the potency of an active constituent may range from 110% to 90%. The expiration date of a product will be determined from the length of time that all of the lyophilized formulation remains within the specified potency limits. There are two

basic methods for determination of the stability of a lyophilized product: accelerated and long-term or real-time stability studies.

Long-Term or Real-Time Stability Testing

Long-term or *real-time stability studies* are conducted under the temperature and humidity conditions specified for storage of the product. At various time intervals, samples are removed and tested to determine the potency or activity of the dried product. Long-term or real-time stability testing extends over a period of years, and the results are used to determine a safe stability period and to assign the expiration date for a given batch.

Accelerated Stability Testing

The rate of the kinetic clock can be *accelerated* by maintaining the dried product at one or more elevated temperatures in a controlled-humidity environment. A typical accelerated test would be performed in an environment that is maintained at an elevated temperature of 40°C and a relative humidity of 50%. Samples are periodically removed from the accelerated study environment, and the potency or activity is determined. The dried formulation is considered stable if there is no significant change in the distribution of potency values over the duration of the test. This type of stability testing is helpful in assessing the presence of any thermal instability in a batch of dried product, but it cannot be used to determine the overall stability of the product and to assign an expiration date. The principal purpose of this form of accelerated stability testing is to correlate the results with the stability of the product as ascertained from long-term studies. A more detailed discussion of using the Arrhenius' equation to determine the stability of a lyophilized formulation is included in Chapter 10.

Cosmetic Properties

The *cosmetic properties* or appearance of a lyophilized product is dependent, in varying degrees, on each step of the lyophilization process (i.e., freezing and the primary drying processes). Examples of the impact that these steps can have on the cosmetic appearance of a *cake* are illustrated in Figure 1.3. In Figure 1.3a, the resulting final cake is uniform in nature. The uniform cake structure is a result of the ice structure that formed during the freezing process. The cake structure shown by Figure 1.3a is representative of the ideal structure of a lyophilized cake where the cake has a spongelike structure, and the cake volume is equivalent to the volume of the frozen matrix illustrated by "B" in Figure 1.1b. The means for forming such an ideal cake structure during the freezing process will be examined in greater detail in Chapters 3 and 7. The formation of the heterogenous structure illustrated by Figure 1.3b will be shown to be dependent on the freezing process.

The impact that the primary drying process can have on the cake structure is illustrated by Figures 1.3c and 1.3d. The conditions under which primary drying is conducted can result in partial collapse of cake structure, as illustrated by Figure 1.3c. The presence of such collapse is generally associated with the primary drying process when the ice-product matrix (see Figure 1.1b) is not in a completely frozen

Figure 1.3. Effect of Freezing and Primary Drying on the Cosmetic Properties of the Cake

Fig. 1.3a   Fig. 1.3b
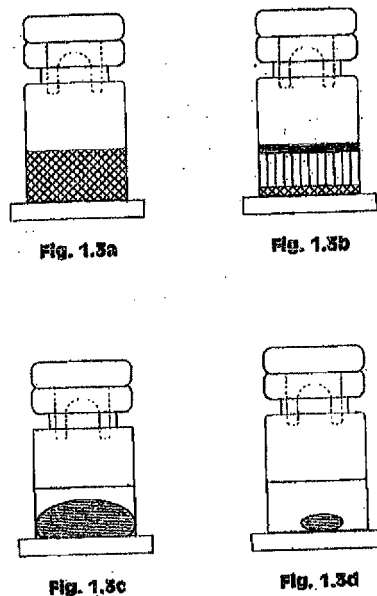
Fig. 1.3c   Fig. 1.3d

Figure 1.3a illustrates a cake matrix as a result of the formation of a uniform matrix during the freezing process. Figure 1.3b shows the formation of a nonuniform matrix as a result of heterogeneous ice formation and the presence of a crust or glaze on the upper cake surface. Figure 1.3c shows collapse of the cake structure as a result of the primary drying process. Figure 1.3d is an illustration of meltback of the product during the primary drying process.

state. An extreme case of cake *collapse* or *meltback* of cake is illustrated by Figure 1.3d. Meltback is a result of the presence of liquid states in the ice-product matrix. The conditions that result in collapse and meltback during the primary drying process will be examined in greater detail in Chapter 8.

Moisture

The main function of the lyophilization has been defined as enhancing the stability of a formulation primarily by the reduction of the solvent system, primarily water, to quantities that will no longer support biological growth or chemical reactions. The quantity of water remaining in the product, in order to achieve the desired stability, is product dependent. The reason for such product dependency is that there are basically two types of water that can be present in the lyophilized formulation.

10

Product Properties

INTRODUCTION

Need for Evaluation of the Lyophilized Product

An evaluation of the final lyophilized product serves to characterize its various physical and chemical properties and the effect that the storage conditions will have on such properties. The evaluation of these properties is, without question, of paramount importance. However, the results of such evaluation should also provide insight as to what effect the initial formulation and the various steps in the lyophilization process may have had on the observed properties of the product. It is not merely a question of what the properties of the final product are but also their source. The relationship between the observed properties of the final product and the materials and the processes that were involved (as described in the previous chapters) can be evident only as a result of careful documentation of the analytical methods and their results. This documentation of the analytical methods and nature of the final product not only verifies batch-to-batch consistency but also provides a vital benchmark that can be an invaluable source of information should a significant change occur in the nature of the lyophilized product.

General Overview of the Chapter

Before embarking on reading this chapter, it will be helpful to the reader to have an idea of why I chose this particular arrangement of the various topics. I feel this chapter should provide the reader not only with methods for product evaluation but also with the benefits derived from understanding the work of other investigators. The reader should understand that I will at times describe and disagree with, rather than ignore, the methods and/or the results of some investigators. The purpose for the critiques of the works of others is not to belittle their contribution to the field of lyophilization but to familiarize the reader with possible pitfalls that may be encountered in selecting an evaluation method or interpreting the results. I am keenly aware that without the works of other investigators, this book would not have been

possible, and for that reason I feel deeply indebted. Consequently, this chapter will consider four major topic areas: physical properties, moisture, reconstitution, and product stability.

General Physical Properties of the Cake

The first section will provide the reader with a description of the general physical properties of the final product. Wherever possible, special attention will be given to the relationship between the observed physical properties of the cake and the composition of the original formulation. It will be shown how selecting the constituents of the formulation can affect a wide range of cake properties (e.g., from temperature effects to physical characteristics that include color, porosity, and structure). It will be shown that even vial breakage has been found to be related to the composition of the formulation.

Moisture

It has been established that the moisture content in a lyophilized product is an important factor that often relates to its physical and chemical properties, i.e., its stability at a given temperature. This section will first consider the different roles that water plays in the final product and will expand on the roles of *residual moisture* or *free* water and the *bound* water that were introduced in the previous chapter. The basic difference between these two forms of water is not in their chemical configuration but in their interaction with the constituents of the formulation. Various means for determining the moisture content of a product will be presented and examined.

Reconstitution

In most applications of lyophilization, with the exception of some food products such as freeze-dried ice cream, a prescribed diluent must be added to the dried product in order to obtain the desired formulation. This process of dissolving the cake by the addition of a diluent is generally referred to as *reconstitution*. The rate and properties of reconstitution of a lyophilized product will be dependent not only on the nature of the diluent but also on the surface area of the cake that results from both the nature of the original formulation and the lyophilization process. Specifications for and means for determining particulate matter in the reconstituted product will be considered, as will the effects of storage conditions. While reconstitution is a destructive test of a lyophilized cake, it represents the key criterion for acceptability of the final product.

Stability

The first part of the definition of the lyophilization process states that . . . *it is a stabilizing process. . .* [1]. For that reason, the last section of this chapter will be devoted exclusively to identifying those properties and contents of the cake that play a key role in determining the stability of the final product. The two basic methods for determining the stability of a formulation will be described and compared. With the increased emphasis on the lyophilization of biotechnology products, special attention

will be given to introducing the reader to aspects that can affect the stability of lyophilized products containing proteins.

PHYSICAL PROPERTIES

The physical properties of a lyophilized product can provide key information regarding the nature of the formulation, e.g., nature and concentration of the constituents and the impact of the lyophilization process. I have, for the sake of clarity, broken down this section into two discrete sections: those cake properties that are mainly associated with the formulation and those that are directly related to the process. The reader is cautioned that observed physical properties may result from a combination of formulation and lyophilization process parameters that may prove difficult to separate and the two topic areas may overlap under some circumstances. I would also like to stress that some of the following physical characteristics, while not appearing as important as potency or activity of the product, should be viewed as fingerprints of the final product and that documenting such fingerprints could be vital in troubleshooting the formulation and the lyophilization process at some later time. One should not ask what can go wrong but rather what steps do we take when it does.
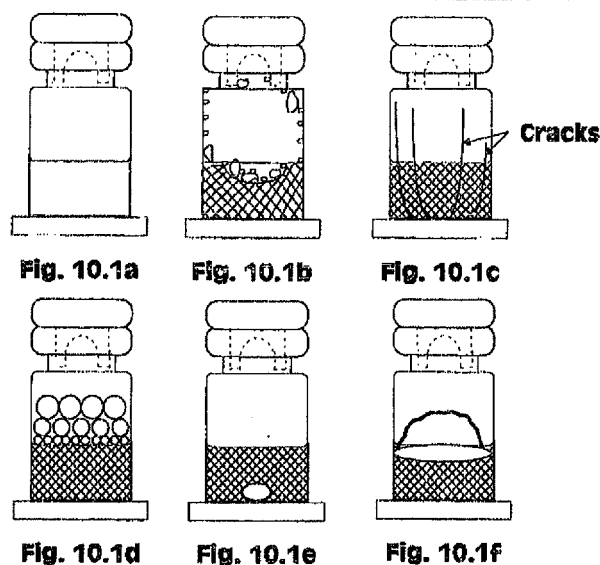
Effects of the Formulation

Transition Temperature

When the temperature of a lyophilized cake is raised, the cake can undergo physical transformation, i.e., shrinkage or liquefaction. The onset temperature for such a transformation is referred to as the *transition temperature* $T_g(w_g)$ [2]. What can often lead to some confusion is that the *glass transition temperature* associated with the freezing process is denoted as T_g' [2], which is for all practical purposes equivalent to the *collapse temperature* (Tc) defined in the previous chapters. Because of the possible confusion between the terms $T_g(w_g)$ and T_g', the transition temperature will be referred to as *incipient melting* and be symbolized as T_a [3]. The onset temperature for T_a has been found to be dependent on the residual moisture content. As the residual moisture in the cake increases, the onset temperature for T_a will tend to decrease [2]. A lowering of T_a can account for the disappearance of the cake during storage, as illustrated in Figure 10.1a. While T_a has been found to be moisture dependent, the reader should not overlook that T_a is also dependent on the nature and quantity of the constituents that make up the lyophilized cake. For that reason, as it was in the thermal analysis of the frozen matrix, there is no simple way to predict and determine the value of T_a for a given residual moisture content. In order to ensure that the cake does not undergo a physical transformation, the value of T_a for the upper limit moisture limit of the product should be established.

Differential scanning calorimetry (DSC) has been used to determine the T_a of a dried product [4]. The difficulty with using DSC to ascertain T_a for a given lyophilized cake is that one must transfer a small sample from the protected environment of the product vial to the DSC pan. In order to prevent an error in the measurement, one would have to know the relative humidity in the product container

Figure 10.1. Product Defects

Fig. 10.1a   Fig. 10.1b   Fig. 10.1c

Fig. 10.1d   Fig. 10.1e   Fig. 10.1f

Additional product defects resulting from the formulation and/or the lyophilization process. Figure 10.1a is an illustration of the disappearing cake resulting from the temperature of the cake exceeding the transition temperature T_g; Figure 10.1b is an illustration of a cake with a poor self-supporting structure; Figure 10.1c is an illustration of vial breakage by the use of mannitol as an excipient; Figure 10.1d is an illustration of frothing or foaming; Figure 10.1e represents an example of partial meltback and Figure 10.1f is an illustration of puffing of the cake.

and match it with the relative humidity in the system used to conduct the product transfer. If the humidities are not equivalent, then a higher transfer environment humidity will result in a false low value of T_g because the moisture content in the sample will be increased. A lower humidity in the transfer environment will result in a lower moisture in the sample and a false high T_g value.

There appears to be, at least at this time, no accurate means for determining the value of T_g while the sample is still confined in the protective environment of the container-closure system. It will be shown in Chapter 12 that as the temperature is increased, there will be considerable outgassing of water vapor from the closure. If the outgassing of water vapor from the closure substantially increases the relative humidity in the container-closure system, then the value of T_g is not a true representation of the dried material obtained from the lyophilization process but some combination of the residual moisture in the product and that moisture outgassed from the closure. T_g is an important property of the lyophilized product; however, accurate means for its measurement awaits development.

Cake Volume

The volume of the cake can be an indicator that the cake was a result of a lyophilization process. For the typical formulation, the volume of the cake will be equal to that of the frozen matrix, like that illustrated in Figure 1.1a [5–8]. It has been my experience that the use of some excipients, such as polyvinylpyrrolidone (PVP) can provide a cake with a proper volume even when primary drying of the lyophilization process was performed at temperatures that exceeded the Tc (see Figure 1.3c). For that reason, if a product was produced by lyophilization, one must exercise caution on making an assessment based solely on appearance (i.e., appearances can be deceptive).

Color

The *color* of the cake, especially in the case of food products, is relative to the structure of the cake. As was emphasized in Chapter 7, the formation of ice crystals will affect the structure of the lyophilized cake.

If frozen rapidly, some products, especially foods, tend to produce a light-colored cake. When the product is frozen at a slower rate, the resulting dried product will usually have a darker color [9].

The color of the final product can also be affected by the conditions during the primary drying process. Lyophilized products tend to be lighter in color than products that haven been vacuum dried temperatures above the Tc [9].

That most lyophilized formulations are colorless does not diminish the importance of observing the shade of the final product. A change in the shade of the product can be a result of a change in the freezing process of the formulation. One should note any relationship between the color or shade of the product and other key properties such as potency of the product, especially during the development stage of the product.

I have occasionally been involved in the lyophilization of colored solutions. In all cases, the color of the initial formulation differed significantly from that of the lyophilized cake. I was often surprised to find that at times little or no attention was given to documenting the color of the initial formulation let alone the resulting lyophilized cake. Such documentation may be quite useful should a problem occur with the quality of the final product. It is true that one can always go back and make a comparison with the retention samples from previous batches, but there would always be the question whether or not the color of the cake had changed during storage.

Texture

The ideal *texture* of a lyophilized cake will be highly porous, and the cake will have a sponge-like appearance. Because of the individual characteristics of each formulation, there is no general rule for an acceptable texture of the cake and each product must be examined with regard to its individual characteristics [10].

The importance of identifying the general characteristics of the texture of the cake is that the texture may be altered either by the freezing process or the primary drying stage [10]. Placement of the formulation on a prechilled shelf (e.g., -40°C) could alter the texture from Figure 1.3a to that of Figure 1.3b. Conducting the

drying process when the product temperature $(T_p) > Tc$ can also result in a change in the texture of the cake [10], as illustrated in Figure 1.3c. It is for these reasons that the observed texture of the cake is an important physical characteristic to document in order to ensure that there is continued quality of the product from batch to batch during manufacturing.

Cake Density

The *cake density* is, in the absence of collapse or meltback during the primary drying process, an indication of the self-supporting structure of the cake. This physical characteristic of the cake will, therefore, be primarily related to the freezing process. For lyophilized products, the cake density is surprisingly low. One means for determining the bulk density of a lyophilized product was described by Fink [12]. This method involved adding a known mass of lyophilized product to a graduated cylinder. The cylinder was then dropped 10 times from a height of 4 cm. The density of the cake was determined from the ratio of the known mass of the cake and the final volume of the cake in the graduated cylinder.

Shrinkage or Collapse

Cake *shrinkage*, more commonly referred to as *collapse*, is apparent when the cake volume is less than that of the frozen matrix, as illustrated in Figure 1.3c. This figure shows that the cake volume is less than that of the original frozen matrix, which is represented by the upper solid line. Collapse, not merely a lack of adherence of the cake to the walls of the container, is an indication of a possible change in the composition of the formulation, improper operating parameters during primary drying, or the presence of excessive moisture.

A simplified pictorial explanation of shrinkage or collapse is illustrated in Figure 10.2 using the analogy of a brick wall. In Figure 10.2a, the frozen matrix is illustrated by a brick wall. The bricks are the dark rectangles, while the clear area represents the mortar, or the region occupied by the constituents and any uncrystallized water. In the lyophilization process, shown by Figure 10.2b, the ice (bricks) is removed by sublimation and the remaining interstitial region (mortar) forms a highly porous system that retains the original volume of wall. If the bricks (ice) are removed while there is still mobile water in the interstitial region or mortar, the result is shrinkage or collapse, as shown by Figure 10.2c.

Shrinkage or collapse can occur as a result of a change in one of the constituents in the formulation (e.g., the active constituent), especially if such a constituent stems from nature or from improper preparation of the formulation. A change in the composition of the formulation can result in a significant change in the thermal properties. Should the Tc of the formulation occur at a lower temperature than that previously determined, then the T_p will be conducted at a temperature, during primary drying, that exceeds the Tc of the frozen matrix. Because $T_p > Tc$, shrinkage or collapse of the cake can occur, as illustrated in Figure 10.2c. Generally when shrinkage or collapse does occur as a result of a change in the thermal properties of the formulation, the shrinkage or collapse of the cake will be apparent throughout the entire batch. Shrinkage or collapse of the cake as a result of a change in composition of one of the constituents in the formulation can be easily prevented by determining the thermal properties of the formulation when first using a new lot

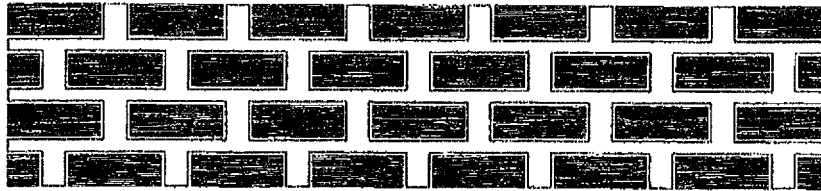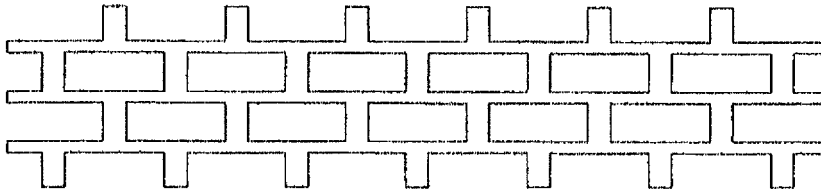## Figure 10.2. The Interstitial Region During Primary Drying
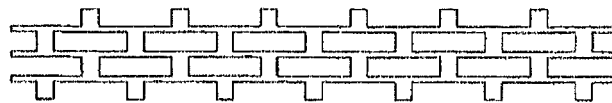
Figure 10.2a

Figure 10.2b

Figure 10.2c

Figure 10.2d

Pictorial illustration of the function of the interstitial region during primary drying from the perspective of a brick wall. Figure 10.2a shows the matrix of ice crystals (bricks), indicated by the dark rectangles, which are surrounded by the constituents and any uncrystallized water (mortar). The wall with the bricks removed (by sublimation) is illustrated by Figure 10.2b and is used to illustrate a lyophilized cake. Figure 10.2c illustrates the effect of removing the bricks while mobile water is present in the mortar, i.e., shrinkage or collapse. Figure 10.2d shows meltback as a result of removing the bricks when the mortar is "wet" and not self-supporting.

of the constituent. If a major change in the thermal properties is observed, then one has the choice of either using a more acceptable lot of the constituent or altering the primary drying parameters to accommodate the change in thermal properties.

The addition of PVP to the formulation can be used to prevent the collapse of the cake. However, shrinkage or collapse can still occur if the concentration of the PVP in the formulation is too low [5]. The increase in the concentration of the PVP in the formulation may prevent shrinkage or collapse of the cake; however, primary drying may be conducted in the presence of mobile water that may compromise the quality or potency of the active constituent. In this sense, the presence of shrinkage or collapse is a warning that the product may not have undergone a lyophilization process. Masking the collapse process with a compound such as PVP may prove counterproductive in the sense that it removes a natural indicator of the state of the drying process.

Shrinkage or collapse of the cake can occur in only a few of the containers or vials that are randomly scattered throughout the batch. The shrinkage is probably not generated by a major change in the thermal properties of the formulation but is a result of a change in the frequency distribution of the heat-transfer coefficient (C_o) of the containers [1]. The collapse is a result of the presence of some containers, because of a broad frequency distribution of C_o values, having significantly higher heat-transfer coefficients than the mean heat-transfer coefficient ($\bar{C_o}$). During the primary drying process, the T_p of these latter products will exceed the Tc, resulting in shrinkage, collapse, or meltback of the cakes. This topic will be considered later in this chapter; but for now the reader is referred to Figure 1.3d and Figure 10.2.

Excessive residual moisture in the cake can also cause shrinkage to occur. In this instance, the shrinkage or collapse may not be apparent immediately upon removal of the vials from the dryer but occur over a period of time. As previously mentioned, the source of the moisture may be a result of outgassing of water vapor from the closures during storage at an elevated temperature.

Pores

As described in Chapter 6, the freezing process generates a matrix of ice crystals and an interstitial region that consists of the other constituents in the formulation. The removal of ice crystals during the primary drying process will then generate a system consisting of pores. While it should be understood by the reader that such a pore system is highly complex and strongly dependent on both the composition of the formulation and the drying parameters, for this discussion the pore will be represented as cylinders like that shown in Figure 10.3. It is further assumed that the cylinders or pores are open on both ends. For pore diameters of the order of 100 Å, an increase in the ratio of P/P_o' (where P is the pressure of the *adsorbate* [e.g., water vapor] above the cake and P_o' is the saturation pressure for the adsorbate at a given temperature T) to values ≥ 0.5 will result in a condensed state of the *adsorbent* [13].

In Figure 10.3, when the R_1 of the adsorbed gas layer becomes equal to the radius of the meniscus of the liquid adsorbate (R_2), the pore will be filled with adsorbate. The total radius (R) will be equal to

Figure 10.3. The Condensation of a Liquid Phase of an Adsorbate in a Pore Having a Radius of R_1 When $R_1 = R_2$, the Radius of the Meniscus of the Liquid Absorbate
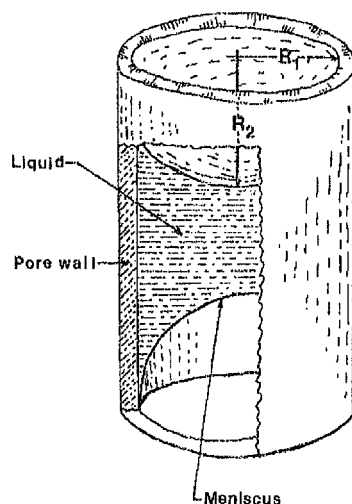
$$R = R_1 + t' \tag{1}$$

where the term t' is related to the thickness of the adsorbed layer.

The *critical volume of liquid* (V_L) in the pores of a cake to induce incipient melting can be expressed as

$$V_L = \frac{PV_a V_m}{R_o T} \tag{2}$$

where V_a is the volume of gas adsorbed on the surface and V_m is the molar volume of the liquid adsorbate.

If it is assumed that the macro pores (i.e., those pores that do not become filled with liquid adsorbate) will have little contribution to the formation of V_L, then the average pore radius $R_{p,T}$ for a pressure P and temperature T to induce incipient melting can be expressed as

$$R_{p,T} = \frac{2V_L}{A_s} \tag{3}$$

where A_s is the surface area of the cake determined by the BET theory (see Chapter 9). A knowledge of A_s will provide a measure of $R_{p,T}$ for a given V_L but will not reveal the frequency distribution of R. If the frequency distribution is relatively broad, the formation of the liquid states, for a given temperature, will occur over a relatively large range of pressures. For a broad frequency distribution of R, the partial pressure of the adsorbate in the system may not be sufficient for the cake to

achieve V_L. However, if the frequency distribution of the pores is rather narrow then the total number of pores containing liquid states may result in a phase transition for the system (i.e., from a solid state to a liquid state). Although this statement offers a plausible explanation for the occurrence of the *disappearing cake* (i.e., a change from a solid to a liquid) in all of the vials of a particular batch of a lyophilized product, there is a clear need for further research in this area.

Crystallization

X-ray diffraction is an excellent analytical method for ascertaining the crystalline nature of a material [10,14,15]. So that others may duplicate the results for lyophilized materials, it is recommended that the wavelength of the radiation used with the diffractometer and the means used for calibration of the instrument be described [15]. Simatos and Blond [10] examined the effects that lyophilization had on various excipients. For example, they were able to show that for a casein–NaCl solution, the NaCl could exist in three states (bound to the casein, free and amorphous, and free and crystalline [10]. After the casein–NaCl cakes were exposed to water vapor an X-ray diffraction pattern showed that the crystalline NaCl was not hygroscopic. The free amorphous NaCl, however, when exposed to the water vapor, was hygroscopic and continued to show a diffused X-ray pattern.

In addition to X-ray diffraction, electron diffraction has been used to characterize the structure of lyophilized cakes [10]. The cake resulting from the lyophilization of a sucrose solution was observed to be mostly amorphous. Results of electron diffraction studies of the sucrose cake did reveal some organization. In some spots of the cake, there were diffraction patterns associated with single crystal, i.e., Laue pattern. The presence of the Laue pattern was never observed in the vitreous portion of the sucrose cake, and the presence of such sucrose crystal nuclei in the cake is not thought to have been induced by the energy of the electron beam. Such nuclei will, upon warming of the cake, promote complete crystallization of the sucrose cake [10].

Structure

The self-supporting structure of a cake is intuitively associated with the solid content of the formulation. Although I did not perform any comprehensive study of the relationship between the solid content of a formulation and the self-supporting structure of the lyophilized cake, experience has shown that, when the solid content becomes less than 2% wt/v, there is a greater risk that the resulting cake structure will not have sufficient strength to prevent the physical breakup of the cake during the drying process. A cake having a poor self-supporting structure is illustrated by Figure 10.1b. The poor self-supporting cake structure appears to be aggravated by the quantity of the fill volume. Large fill volumes of low solid content formulations, perhaps a result of the low solubility of the active constituents, will have a greater tendency to exhibit a poor self-supporting structure than the same formulation with a lower fill volume.

In some instances, the loss of product from the container, as a result of a poor self-supporting structure can create serious problems involving not only a loss of product but also a hazard to the operating personnel and the facilities. The portion of the cake that leaves the vial is often in the form of a fine powder that is not always

visible in the chamber when the door of the dryer is opened. This fine powder can present several problems.

Operating Personnel. In case there is an apparent loss of cake from the containers, operating personnel should be equipped with the appropriate gowns and breathing apparatus, in compliance with the requirements specified by the Materials Safety Data Sheet (MSDS) for each of the solid constituents that make up the formulation. Special procedures and training of personnel will be required to ensure personnel are not exposed to the dust during removal of the gown. Gowns that may be contaminated should be disposed of in accordance with the instructions specified in the MSDS. My major concern is that the information contained in the MSDS may not have been completely documented. This will be of particular concern for those individuals engaged in the development of the lyophilization process. Because these personnel are working with relatively small amounts of product, personnel safety issues can often be overlooked or ignored. It is unfortunate but often true that *familiarity breeds contempt.*

Facilities. In a facility that contains a clean-air handling system, an increase in particle count could be an indication of the entry of dust particles into the manufacturing area. However, in those facilities not requiring such monitoring, as in the case of some diagnostic products, there may be a buildup of dust in the room that could be harmful not only to personnel but in some cases to the electronic equipment.

Environmental. If it is known that significant quantities of the product are entering the dryer, then the condensate from the condenser and the water used to rinse the dryer may require treatment prior to being discharged into the public sewage system. Such a need for expensive wastewater treatment could have been avoided had adequate consideration been given to the manufacturing of the product during the formulation development stage.

Product. Although each of the above effects of poor cake structure is of concern, perhaps the greatest concern is with the product itself. A key problem is that any loss of cake from the container will reduce the potency of the drug in that container. With no supporting data, it is my opinion that the amount of loss from product will vary from vial-to-vial. One may be tempted to compensate for the loss in potency by increasing the potency to the upper-limit allowed by the USP for the particular drug, a concept similar to the rationale of keeping extra sheep in the pen to compensate for those that may escape through a hole in the fence.

Perhaps the most difficult problem associated with dust generated by a poor self-supporting cake structure is the dust that will be on the outside of the container. Then the question arises, *How does one ensure, especially if the product is one that should not come in contact with the skin, that such product containers can be safely handled once they are in the public domain?* One possible solution would be to caution the user to handle only while wearing gloves. Such a label would not instill confidence in manufacturing practices.

The solution to these problems is relatively simple: during the initial formation of the product, make certain that the solid content is $\geq 2\%$ wt/v.

Cake Strength

The strength of a cake is a measure of its ability to withstand stress and is a gauge of its durability. This physical property of the cake is related to the nature and the concentration of the excipient(s) in the formulation [5]. Except in the case of an electrolyte such as NaCl, PVP is an excellent excipient to improve the strength of a lyophilized cake [5]. PVP's capability to increase the strength of a cake is attributed to its elastic and thread-like binding properties arising from polymeric characteristics [5]. It is not certain if such binding by PVP occurs during the freezing or the primary drying process.

A means for determining the strength of a cake has been devised [12]. In this method, the cake is transferred to a closed glass container. The container is then placed on a spring-suspended table and shaken by means of an eccentric drive mechanism. The drive mechanism generates 213 horizonal movements per minute and the same number of vertical movements per minute with an amplitude of 4 cm. After shaking for 30 min, the density of the cake was determined in the same manner as described above [12]. The percentage in the reduction in the density of the cake is considered to be proportional to the original volume of the specimen [12] and is associated with the mechanical strength of the cake.

Fractureability

The *fractureability* of a cake is a measure of the force necessary to break or crack the cake. A method by Bashir and Avis for determining the fractureability of a cake has already been described in detail in Chapter 2 [5]. These authors found that cakes made with excipients like mannitol and PVP required a considerable force to break. For example, cakes formed by the lyophilization of 0.2 M mannitol solution had a fracturability of 88 g ± 19.6 g. Such a large standard deviation would suggest that the fracturability of a cake is best viewed as a qualitative rather than a quantitative analysis of the cake structure. Forming a suitable cake with mannitol required the addition of NaCl and even those cakes were rather fragile.

Vial Breakage

The composition of a formulation, in combination with the lyophilization process, can result in vial breakage like that shown in Figure 10.1c. The breakage of vials as a result of using mannitol as an excipient was first reported by Jennings [16]. Williams et al. [17] first demonstrated that actual vial breakage occurred during the warming of a frozen mannitol matrix. When vial breakage did occur, these authors observed an increase in the electrical resistance of the system. The increased electrical resistance was found to be associated with an ice recrystallization process. Rapid cooling of the mannitol formulation produced a matrix that formed a system that could result in ice recrystallization. When the mannitol formulation was cooled slowly, a matrix that did not result in ice recrystallization was formed and vial breakage was not observed. It was later established that the breakage was associated with an exotherm resulting from the recrystallization of the mannitol [18].

Effects of the Lyophilization Parameters

The properties of the cake that are a direct result of the lyophilization process or storage conditions will be considered here. The reason for separating those properties that are primarily associated with the formulation and those associated with the lyophilization process is to provide the reader with a basis for identifying a possible source of a defective product. The reader is again advised, as demonstrated above, that the observed properties may be related to either the formulation, the lyophilization process, or a combination of both.

Potency

For a loss in potency in the final product, one of the first processes to consider is the freezing process. The reason, as stressed in Chapter 7, is that during the freezing process, ice crystals are formed, and there are major changes in the concentration of the excipients. In the case of biological products such as vaccines, the freezing process may cause a loss in the titer of the vaccine. This loss in titer can be a result of a change in *p*H, a major change in the concentration of gases that could prove harmful to the virus, or the removal of water that results in the denaturation of the proteins associated with the virus [19,20].

Matrix Structure

Nei and Fujkawa [21] used optical microscopy to gain an insight into the mechanisms involved during the freezing process of a formulation. Thin specimens of the formulation were sandwiched between two cover glasses, and the temperature was then lowered. As freezing of the formulation occurs, one obtains a pictorial account of the growth of the frozen matrix. While such pictorial accounts are often dramatic to observe, these authors expressed some reservations about whether the freezing in the thin layer specimen was truly representative of what actually occurred during the freezing of bulk materials [21].

Bilayer Structure

Situations may occur where certain constituents in the formulation are found to be incompatible because of possible chemical interactions. Such chemical interactions can result in a loss in potency before and during the freezing process.

For example, Haby et al. [22] reported that a product required the nitroimidazole ligand known as BMS 181321 to react with technetium-99m. The BMS 181321 ligand was obtained from the reduction of a ligand identified as BMS 181032 by a reduced form of technetium in the presence of stannous chloride ($SnCl_2$). The problem was that the ligand BMS 181032 was found not to be stable in the presence of stannous chloride. These investigators solved this dilemma by forming a bilayer matrix.

The bilayer matrix was formed by first freezing a given volume of the ligand BMS 181032 formulation containing sulfated β-cyclodextrin solution to -50°C and maintaining this temperature for a period of 1 hr. A given volume of $SnCl_2$ was added to the container, and the entire system was again frozen to -50°C.

The bilayer frozen matrix was then lyophilized, and the result was a cake having two separate layers. Lyophilization of the formulation frozen in a bilayer configuration resulted in a final product in which the ligand BMS 181032 retained > 90% of its initial concentration. The ligand still retained 85% of its original potency even after 4 weeks of storage at 40°C [22].

Froth or Foam

The final product cake may contain a *froth or foam* on the upper surface like that shown in Figure 10.1d. In general, frothing or foaming is undesirable because it provides a system that may have a different composition than that of the main formulation and could lead to interactions, such as the aggregation of proteins. Even if the frothing or foaming is found to have no deteriorating effect on the quality of the lyophilized product, its presence does not instill the same degree of confidence in the user as does a well-formed matrix, like that illustrated in Figure 1.3a.

The formation of this froth or foam on the upper surface of the cake can stem from two possible sources, one of which is the filling of the vials. In some formulations, notably those formulations containing proteins, the rapid dispensing of the fill volume into the vial can produce a foam on the surface. The foam will tend to persist during the freezing process and will be present in the final lyophilized cake.

A second source of foaming or frothing could stem directly from the freezing process. If the formulation contains significant quantities of dissolved gases, particularly CO_2, the gas will be concentrated in the interstitial region where it can reach saturation. If the interstitial region is still in a liquid state, the increased gas pressure can deform the matrix and provide a path for release of the gas. The release of the gas results in the formation of a surface that is characteristic of frothing or foaming. Frothing or foaming will tend to be more prevalent during the freezing of natural food products than in pharmaceutical or biotechnology formulations. If foaming or frothing is a problem that occurs during the freezing process, the gas concentration can be decreased by *sparging* the formulation with a gas that has a low solubility, such as nitrogen or helium. Even with sparging, one must exercise caution that excessive foaming does not result in the denaturation of the protein constituents in the formulation.

Cake Strength

The freezing process can also impact the cake strength. It has been found that quickly frozen products will tend to have a higher cake strength, as described in the previous section, than products that are frozen slowly [12]. While cake strength can be affected by the freezing process, the solid content of the formulation (i.e., wt/v) will still be the prevailing factor for the cake structure.

Crust or Glaze

The formation of a *crust* or *glaze* on the surface of the cake is strongly dependent on the nature of the freezing process. As illustrated in Figure 1.3b, the formation of a heterogenous matrix is a major factor in forming a crust or glaze during the freezing process. In particular, a crust is generated by the formation of large ice crystals that tend to concentrate the unfrozen formulation and push the solutes to the top surface.

Such cake surfaces can affect the transport of water vapor from the product and may contain key constituents of the formulation. Dawson and Hockley [23] used scanning electron microscopy to examine the glaze or crust on the surface of the cake. These authors were able not only to examine the structure of the surface layer but also to determine its composition. For example, in one case they found that the crust contained a protein constituent. They reported that the compound was not uniformly distributed throughout the crust but was more concentrated in the upper one-fifth of the crust. These results are of major importance because the composition of the crust may vary significantly from the composition of a homogeneous cake. The ability to identify the composition of a particular region of the cake using scanning electron microscopy offers an important analytical tool for determining not only the structure but also the composition of a lyophilized cake. It is unfortunate that these authors did not elaborate on the analytical technique that was used to ascertain the composition of the crust.

Collapse

The most prevalent impact of the primary drying process is on the nature of the cake structure of the final product. While the thermal properties of the formulation are an important factor, T_p with respect to Tc will have a major impact on cake structure. If $T_p > Tc$, then the presence of mobile water in the interstitial region can lead to *collapse* of the cake, as illustrated in Figure 1.3c or in Figure 10.2c. The degree of collapse will be related to the magnitude of the difference $(T_p - Tc)$. As $T_p - Tc$ increases, the volume of the cake will decrease, as illustrated by comparing Figure 10.2b and 10.2c. With the assumption that Tc remains fairly constant, the magnitude of $T_p - Tc$ will be directly related to the chamber pressure (P_c) and the shelf-surface temperature (Ts).

The reader should be aware that collapse can not only cause a cosmetic change in the appearance of the cake but also can be responsible for a loss in potency. By using scanning electron microscopy of the cake after the completion of the primary drying process, Dawson and Hockley [23] were able to observe aggregation of a protein constituent in a cake exhibiting a collapse structure. Therefore, collapse should not be viewed as merely a cosmetic defect but as a condition that can lead to a reduction in the potency of the final product.

It is hoped that the reader will realize the danger of not measuring T_p during the lyophilization process. By determining that the T_p was approaching the known Tc of the frozen matrix, one could assume that there was an error in the measurement of the T_s or the P_c. If the temperature readings were correct during the freezing process, one would be justified in believing that such measurements are still within specification, and it is the pressure gauge that, for whatever reason, is reading a false low. By adjusting the pressure control system to bring the T_p within the specified temperature range for the primary drying process, one could prevent the possible loss of a batch of product and have the pressure gauge calibrated before attempting to lyophilize another batch of the formulation.

Meltback

Meltback can be viewed as an extreme case of collapse in which liquid states exist in the interstitial region during the primary drying process. In this instance, the

interstitial region is no longer self-supporting, and collapse occurs to a point where there is little cake structure, as illustrated by Figure 10.2d or no cake structure as shown by Figure 1.3d. As with collapse, meltback not only produces a cosmetically unacceptable product but can result in aggregation of the constituents to lower the potency to unacceptable levels. There are basically two forms of meltback.

Partial Meltback. *Partial meltback* occurs when the temperature of the shelf surface is increased to commence the secondary drying process prior to completion of the primary drying process. With the increase in the shelf temperature, the result is that $T_p > Tc$, a small quantity of frozen matrix located near the center bottom of the vial forms a liquid state, and the small region of the cake, like that shown by Figure 10.1e, undergoes meltback.

If partial meltback occurs throughout the entire batch, then the problem is associated with an improper lyophilization process or an error in one of the major process control transducers. An improper lyophilization process will be evident by a T_p not approaching the T_s for a specified period, which indicates an incomplete primary drying process. The problem can be corrected by allowing T_p to approach T_s for some specified period of time. If the frequency distribution of the C_0 values for the vials is known, then, from the standard deviation, one can obtain the estimated additional time for maintaining T_p at approximately T_s in order to ensure that all of the product in containers have completed the primary drying process [1].

Should partial meltback occur in a small number of vials scattered throughout the entire batch, then the problem is again associated with the frequency distribution of the C_0 values of the vials. In this instance, however, it will be the vials in the frequency distribution that have C_0 values that are significantly lower than the mean $\bar{C_0}$ value of the vials. To correct this problem, the primary drying process will have to be extended to include those vials with the lower C_0 values.

Total Meltback. When *total meltback* occurs throughout the entire batch, then all of the vials have a T_p that exceeds Tc throughout the major portion of the drying process. This condition can be corrected by either (1) lowering the P_c while maintaining the same T_s; (2) decreasing the T_s while maintaining the same P_c; or (3) decreasing both the T_s and the P_c so that T_p will be lower than Tc during the period of time when frozen matrix is present in the vials. Except for option 1, revising the process parameters (T_s and P_c) to lower the operating T_p will tend to prolong the primary drying process.

Should total meltback occur in a small number of vials scattered throughout the entire batch, the problem is once again associated with the frequency distribution of the C_0 values of the vials. In this instance, however, it will be the vials in the frequency distribution that have C_0 values that greatly exceed the mean $\bar{C_0}$ value of the vials. To correct this problem, the drying process will have to be slowed down so that the temperatures of the product in the vials having the higher C_0 values will not generate T_p values that greatly exceed the Tc.

Puffing

Puffing is a cake defect (illustrated by Figure 10.1f) in which the upper surface of cake expands during the initial portion of the primary drying process. The reason for the expansion of the cake surface is incomplete freezing of the formulation prior

to lowering P_c to commence the primary drying process [24,25]. Since the puffed portion of the cake stemmed from an unfrozen portion of the matrix, this material should be considered as being a result of partial meltback, even though it is associated with the top of the cake rather than the bottom. If the formulation contains protein constituents, then one should be concerned with aggregation and the possible formation of particulate matter. In addition, aggregation of the protein can result, depending on the fill volume, in a significant reduction in the potency of the final product. The loss in potency resulting from puffing will be more prevalent with formulations involving low fill volumes.

Browning

When cakes having carbohydrates and ascorbic acid as constituents are exposed to excessive heat during the secondary drying process the results can be a discoloration or *browning* of the cake [9]. Although browning is dependent on time and temperature, it is an irreversible reaction. While discoloration of the cake is cosmetic, browning can affect other product properties, such as the potency of the active constituent.

Effects of Storage

The underlying function of lyophilization is to serve as a stabilizing process; nevertheless, lyophilized products can undergo physical and chemical changes during storage. Some of these changes can be beneficial, while others can have a deteriorating effect on the potency of the active constituent.

Effect of Crystallization. Sucrose is often used as a major constituent in a formulation. When such a formulation is lyophilized, the sucrose will tend to be in an amorphous state. Amorphous sucrose has a high affinity for moisture. Because of sucrose's high affinity for moisture, it is often difficult to achieve low moisture content in the formulation. Yet when the amorphous sucrose is stored at an elevated temperature for a specified period of time (e.g., 60°C for 1 month), crystallized sucrose that has a relatively low affinity for moisture is formed [19]. Therefore, the conversion of sucrose from an amorphous to crystalline state will limit any further increase in moisture resulting from the presence of sucrose.

Crystalline mannitol can be formed during the lyophilization process and requires no thermal treatment after lyophilization. But the presence of crystalline mannitol in the lyophilized cake causes a major reduction in the potency of the protein erythropoeitin (EPO) after storage at 4°C for only 6 months [19].

Browning Occurring During Storage. While the previous two examples illustrate how crystallization of an excipient may prove useful in some instances and harmful to active constituents in others, other constituents may, during product storage, produce reactions that can have an impact on the active constituent. Consider a cake whose composition consists of a protein, sucrose, citric acid, and dibasic sodium phosphate. If this system were stored at 60°C for 1 month, there would be a decrease in *p*H upon reconstitution. At lower *p*H values, sucrose hydrolyses into fructose and glucose. The resulting glucose reacts chemically (browning reaction) with the protein constituent [19].

A similar reaction occurs during the storage of dried blood plasma. A chemical reaction involving glucose and protein amino groups, which results in the formation of glucose amides, gives the dried blood plasma a brown color [26]. Even dried blood plasma stored at 20°C and at relative humidities ≥ 7% can, after some period of time, produce a brown color [26].

The key point for the reader to realize is that just because a formulation has undergone the lyophilization, it does not mean that physical and chemical changes cannot occur during the storage of the product. By understanding the nature of these changes and how they impact the active constituent, one can take appropriate steps to protect the active constituent by changing either the constituents in the formulation or the conditions under which the product is stored.

MOISTURE

The moisture content has been long recognized as important because of the major role that it plays in determining the stability of a lyophilized product. In 1949, Flosdorf [27] reported that when a lyophilized formulation containing diphtheria antitoxin had a moisture content of about 0.5% by wt, there was negligible loss in the potency after 3 years of storage. Yet when the moisture content of the latter formulation was increased to between 5% and 8% there was an appreciable loss in potency after just 6 months of storage. In 1950, Lea and Hannan [28] found that the moisture content was responsible for the chemical reaction involving the amino group of casein and glucose. At low moisture levels in the cake, the interaction between casein and glucose was found to be negligible. Thus, it is important to be able to equate the moisture content of a lyophilized formulation with its stability.

The remaining portion of this section will be devoted to a discussion of the nature of the various roles that water performs in the lyophilized cake and a description of analytical methods for determining the moisture content.

Residual and Bound Water

Residual Moisture and Free Water

Free water, sometimes referred to as surface water, freezable water, or residual water [29–31], is defined as water that obeys the desorption isotherms that were illustrated by Figures 9.4 and 9.5. For clarity sake, I will use the term *residual moisture* instead of free water. The principal effect of the residual moisture content on the lyophilized product, as stated above, is to alter the product's stability. Excessive residual moisture will tend to reduce the shelf life of the product [27,28], however, the addition of water has been found to enhance the stability of some lyophilized products [32]. The actual residual moisture necessary to achieve stability will vary from product to product depending on the nature of the individual constituents [25].

Dependence on Surface Area

The adsorption isotherms for water vapor on the surface of a lyophilized cake have already been covered in the previous chapter. The quantity of moisture on the surface was shown to be dependent on the key factors of the lyophilized product.

Relationship of Lyophilization Process and Reconstitution

The following is a brief discussion as to the effect the various lyophilization processes can have on the reconstitution process.

Freezing Process. The freezing process can affect the reconstitution process by the nature of the frozen matrix. For a homogeneous cake structure like that shown in Figure 1.1a, reconstitution could be rapid and complete in a relatively short period of time. In the case of the formation of a heterogeneous cake, as illustrated in Figure 1.3b, the formation of the crust could also cause aggregation of the protein and result in an extension of the reconstitution process. In the case of blood plasma, that the denaturing of lipoprotein occurs during the freezing process has long been suspected [24].

Primary Drying Process. The primary drying process can have the greatest impact on the reconstitution of the product. Collapse of the cake, as illustrated by Figure 10.2, can certainly prolong the reconstitution process, especially for serum-based products. Also *puffing*, as illustrated by Figure 10.1f, can cause aggregation of proteins that would prolong or even prevent complete reconstitution of the product. However, it is the production of meltback during the primary drying process that can have the most serious effect on reconstitution properties. The meltback of a formulation containing protein compounds can result in a material where the interaction between molecules is such that an almost insoluble compound is formed. For such a product, reconstitution could be even a matter of days, and it is possible that the potency of the resulting solution would not meet USP specifications.

Secondary Drying. The complete removal of moisture from a formulation containing proteins in extending the reconstitution time [26]. Excessive aggregation of the protein can lead to the formation of an unacceptable opalescent solution upon reconstitution [47].

Effect of Storage

During storage, blood plasma can form a brown color as a result of the formation of glucose amides. Depending on the quantity of the plasma involved in the browning reaction, the time required for reconstituting the plasma can be prolonged, or the solubility of the plasma is limited. Lyophilized plasma that has been stored for years but did not show signs of the browning reaction was found to have good reconstitution properties. The browning reaction can be inhibited by a reduction of the residual moisture content of the plasma and by lower storage temperatures [26].

Specifications for Particular Matter

The particulate matter in reconstituted lyophilized product is an important consideration. When such particulate matter is present in the reconstituted product in sufficient quantities, it can render the final product unacceptable, even if all of the other properties of the formulation meet USP specifications. The following will be a brief overview of the sources of and the requirements and means for determining particulate matter in a reconstituted formulation.

USP Limits for Particular Matter for Injectable Solutions

The following are the USP limits for particulate matter.

- *Large Volume:* The USP limits for solutions > 100 mL is fifty 10 μm or larger particles per mL [48].

- *Small Volume.* The USP limits for solutions $\leq$ 100 mL is a total of 10,000 particles not exceeding 10 μm [48].

Sources of Subvisible Particles

Extrinsic Particles. Extrinsic subvisible particles can enter the lyophilized formulation from various external sources, such as the production atmosphere, the manufacturing equipment (e.g., the freeze-dryer), a filter, the filling devices, and the container and closure system [48].

Intrinsic Particles. Intrinsic subvisible particles are particulate matter that stems directly from the formulation or the lyophilization process. Such particles can be generated as a result of freezing a supersaturated solution, generated during storage of the product, or can stem from the selection of a surfactant (e.g., pluronic F-69) [48].

Although there is no supporting evidence at this time, I would not be surprised to find that intrinsic subvisible particles can stem directly from the lyophilization process (i.e., during the freezing process when there is a large change in the concentrations of the various excipients and the formation of a crust or glaze on the upper surface of the product). These latter layers may appear to reconstitute, but a portion of them could be denatured to such an extent that they will remain insoluble. Puffing and partial meltback during the primary drying process could all be sources of such particulate matter. Exothermic reactions occurring during secondary drying and the production of a hazy solution [1] could represent process-related sources of these intrinsic particles. There is little doubt that there is a need for additional investigations into the role that the lyophilization process plays in the generation of intrinsic subvisible particles.

Measurement of Particulate Matter

The following is a general description of a method for determining particulate matter in a reconstituted formulation.

Reconstitution. The formulation is first reconstituted using a particle-free diluent. A particle-free diluent is defined as a solution in which there all particles are less 10 μm [48]. It is important the reconstitution be conducted in accordance with the manufacturer's instructions.

Instrumentation. The determination of the particulate matter in a reconstituted formulation requires the use of a HIAC instrument (i.e., an instrument that determines the particulate matter using the light obstruction principle). A given volume of the reconstituted solution passes by a window that is illuminated by a parallel beam of light. The flow of the liquid is controlled so that particles will pass by the window

singly. A reduction in the intensity of the light reaching the photomultiplier tube results from the light scattered by the particle and is recorded as a signal. The amplitude of this signal will be proportional to the area and, hence, the diameter of the particle [48].

Additional Tests of the Reconstituted Formulation

The reconstitution of a lyophilized formulation should result in a solution that mimics the original formulation. Besides the obvious determination of the concentration of the active constituent or potency, other tests should be performed to ensure that the composition and concentration of the other constituents in the formulation are within the established range of the original formulation as outlined in Chapter 2. In order to avoid being repetitious, the following is merely an outline of the various characteristics of the formulation that were suggested in Chapter 2. For more details regarding these characteristics, the reader is referred to Chapter 2.

Colligative Properties

A significant change in the colligative properties would be an indication that the original formulation has undergone significant chemical changes as a result of the lyophilization process. For example, a difference in the freezing point depression of water between the original and the reconstituted formulation would be an indication of a major change in the composition of the formulation. If the original formulation contained sucrose, and there is a decrease in the depression of the freezing point of water with respect to the original formulation (i.e., the freezing point of water occurs at a lower temperature), then it is possible that during the freezing process there was a lowering of the pH that caused the sucrose to dissociate and form glucose and fructose. Testing the solution for the presence of glucose would confirm that such a reaction had occurred. An increase in the freezing point depression of water (i.e., the freezing point depression of water occurs at a higher temperature) would indicate some interaction between the constituents, and tests should be conducted to determine the concentration of each constituent in the reconstituted formulation.

Concentration Properties

While several test methods were suggested in Chapter 2, a simple check of the refractive index of the reconstituted formulation would be useful in determining if there has been a change in the concentration and composition of the formulation as a result of the lyophilization process. While there may not have been a change in the colligative properties, a change in concentration and composition of the constituents may have occurred. A measure of the refractive index would confirm that there was no significant change in the concentration and composition of the original formulation as a result of the lyophilization process.